IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Xiaozhen Xie, Ph.D.
Art Unit	:	1646
Applicants	:	Michel Christian Morre, Brigitte Assouline, Pierre Cortez, Anne Gregoire
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For	:	IL-7 Drug Substance, Composition, Preparation and Uses

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

DECLARATION OF MICHEL MORRE PURSUANT TO 37 C.F.R. §1.132

Sir :

I, Michel MORRE, residing 67 rue National, 92100 Boulogne- Billancourt, FRANCE, declare and Say:

I am a citizen of France.

I am a Doctor in Science Veterinary Medicine, Master in Science in Physiology and Biochemistry and was a post doctoral fellow at the Massachusetts Institute of Technology (MIT), Cambridge, MA USA. I have a cumulative experience of 34 years in research and development within the pharmaceutical industry.

I am now the CEO of CYTHERIS (the assignee of the above-identified patent application) and an inventor of the invention claimed in the above-identified patent application.

I personally supervised the experiments which led to the characterization of the claimed IL-7 conformer which, when present in pure form, shows satisfactory therapeutic activity without undesired immunogenic activity.

I have read the Office Actions issued by the U.S. Patent Office in this matter, references cited in the rejections, responses filed by counsel and the as-filed specification for the above-referenced patent application and I have the following comments and statements.

This declaration provides additional data demonstrating that a very high purity of the correct IL-7 conformer (corresponding to the claimed composition of matter) is necessary to

minimize or avoid immunogenicity and this data augments that found in the as-filed specification within the Examples.

Analytical methods have been used to detect improperly folded/aggregated IL-7 conformers and other impurities that arise from the standard production of IL-7 in recombinant host cells (either eukaryotic or prokaryotic host cells). These product impurities arise from the improper or incomplete refolding of IL-7. Improper and incomplete refolding of recombinantly produced IL-7 can result in self aggregation arising from covalent or non-covalent bonding within and between the recombinantly produced IL-7 molecules. Extremely low amounts of these impurities are sufficient to trigger anti-IL-7 immunogenicity.

1. Purification and analytical steps:

Our practical experience with IL-7 expression in a CHO eukaryotic host cell showed us that the majority of the IL-7 protein expressed in the supernatant before any purification is not the correctly refolded protein but a complex mixture of various unfolded, partly unfolded, wrongly refolded or intermolecular bridged molecules. In our own experience, this was also the case for expression in E. coli or HEK-293 cells implying that this should also be the case in other cell lines such as COS or BHK. Similarly, it would also be our expectation that recombinant IL-7 produced according to the teachings of Ho et al. (U.S. Patent No. 5,714,141) or Namen et al. (U.S. Patent No. 5,328,988) would also result in the production of a complex mixture of various unfolded, partly unfolded, wrongly refolded or intermolecular bridged molecules.

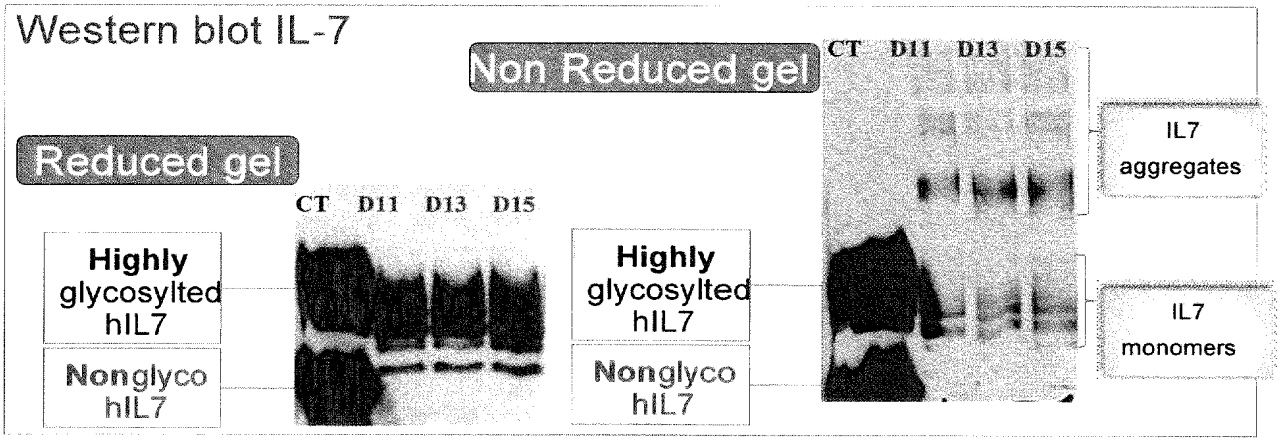
Purification via reverse phase HPLC, classical electrophoresis analysis, and testing in bioassays reveals that a significant amount of contaminant remains present in recombinantly produced IL-7. These contaminants are detectable by a complex technological approach using: (i) SDS gel protein overload and silver staining, (ii) aggregate detection by light scattering, (iii) peptide map analysis before denaturation of the disulfide bridges or (iv) testing immunogenicity of the homologous IL-7 in corresponding species (simian IL-7 in monkeys, human IL-7 in patients).

The following steps, described in great detail in the Examples of the present application, were carried out:

A- Construction and Expression of optimized human (h) and simian (s) IL-7-coding nucleotide sequences
B- Fermentation of E. coli producing recombinant IL-7
C- Fermentation of HEK-293 or CHO cells producing recombinant hIL-7
D- Purification of recombinant IL-7 product Expressed in E. Coli
E- Purification of recombinant human IL-7 product Expressed in HEK-293 or CHO cells
F- Product controls and specifications (Peptide map by Mass spectrometry MALDI-TOF, SDS-PAGE overload and silver staining, light scattering)
G- In vitro biological activity assay of recombinant IL-7
H- Assays for detection of antibodies to recombinant IL-7 in serum

2. Glycosylated IL-7 from supernatant of recombined CHO cell does not systematically refold as a correct monomer

IL-7 Western blot analysis of samples from Day 11, 13 and 15 of a CHO cell culture supernatant from a 200L bioreactor, loaded on a reduced and a non reduced SDS PAGE gel, display a complex pattern of aggregated (high MW bands), unfolded (band shift), incompletely and completely refolded forms of recombinant IL-7.



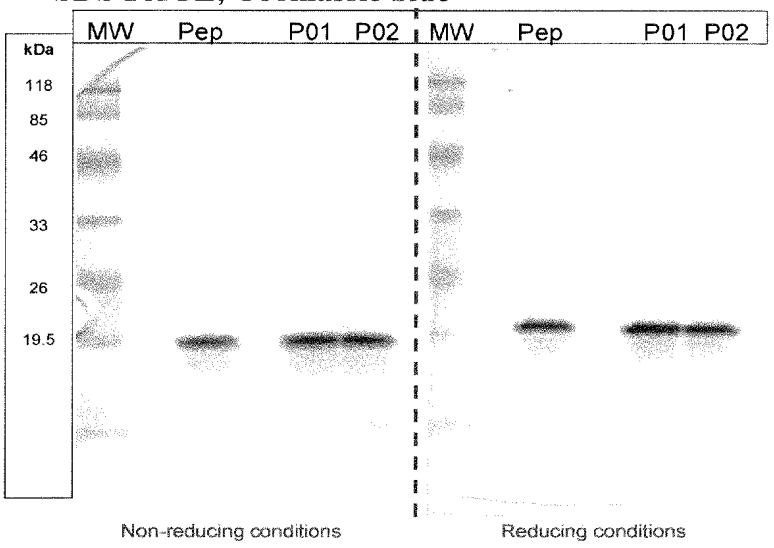
Reduction of the protein to open the disulfide bridges improves protein homogeneity by SDS electrophoresis, reflecting the very complex isoforms linked to various disulfide bridging patterns in the crude IL-7 containing supernatant.

3. Classical SDS page with Coomassie blue does not detect contamination with improperly folded IL-7

Analysis by classical SDS-PAGE and Coomassie blue staining does not detect small amount of incompletely refolded IL-7 products. However, analysis by SDS-PAGE using silver staining of an overload of highly concentrated IL-7 reveals the presence of incompletely refolded IL-7 molecules in a commercial source of r-hIL-7 (Pep) and in P01 which contain residual impurities. PO1 corresponds to the first batch of recombinant IL-7 we produced (and which exhibited substantial immunogenicity in a human patient to whom it was administered).Coomassie blue staining does not show any contaminants with slightly shifted bands on the non reduced side of the gel. Massive overload on silver stained gels allow detection of some incompletely or wrongly refolded IL-7 with a different apparent molecular weight (MW).

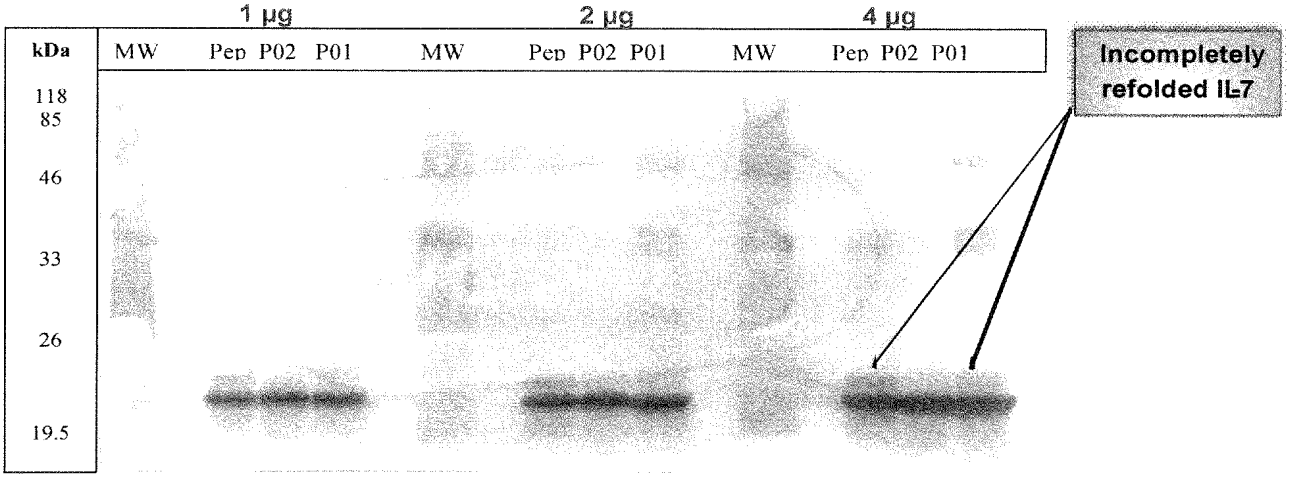
The small bands corresponding to improper/incomplete refolding disappear after full reduction of the "di-cysteine bridges" (right side).

SDS PAGE, Coomassie blue

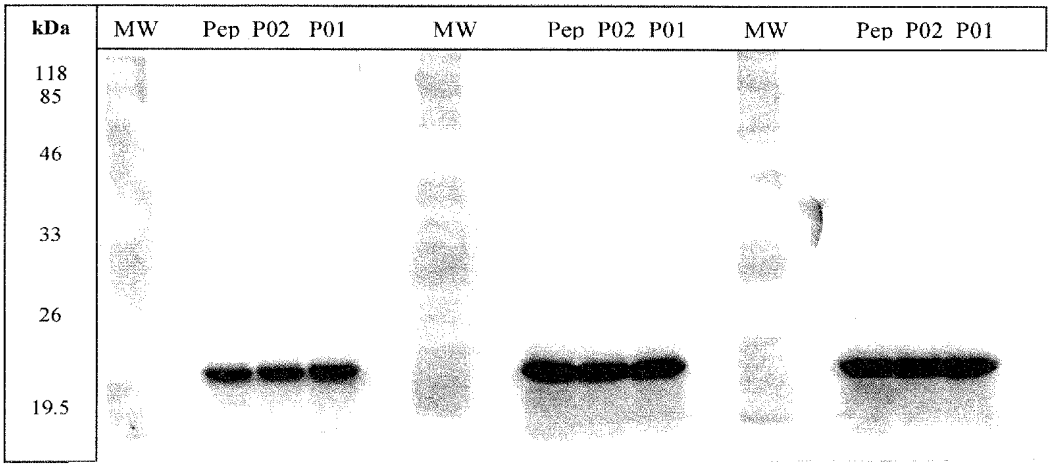


MW : Molecular Weight
Pep : Commercial r-hIL-7 batch
P01 : First r-hIL-7 GMP clinical batch
P02 : Second r-hIL-7 GMP clinical batch

SDS PAGE, Silver Stained



Non-reducing conditions



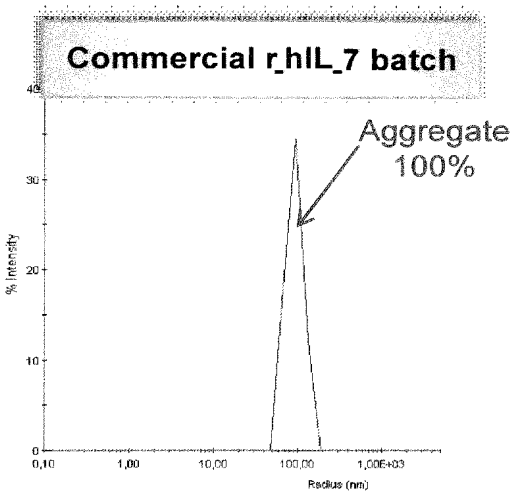
Reducing conditions

4. Insufficient purification and refolding of the non-Glycosylated IL-7 leads to covalent or non covalent aggregation.

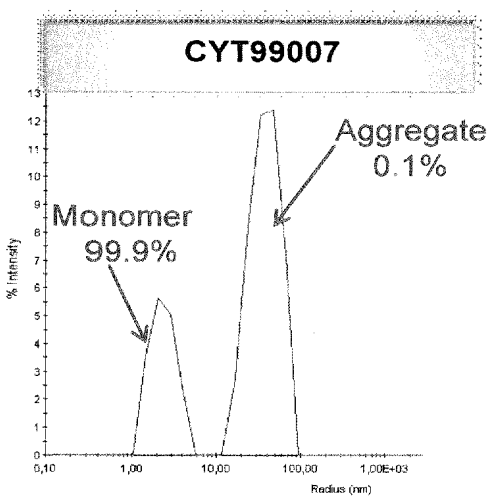
Unfolded or incompletely refolded IL-7 molecules lead to intermolecular aggregation via both covalent and non-covalent interactions. This aggregation exposes hydrophobic regions and reveals new antigenic epitopes which participate in the induction of unwanted anti-IL-7 immunogenicity.

A commercial source of r-hIL-7, purified using a classical process, is more susceptible to formation of intermolecular aggregates as compared to CYT 99007 (*E. coli*-derived recombinant human IL-7, purified using the disclosed process and controlled by appropriate bioanalytical methods to check for product purity).

Percentage of Aggregated IL-7 contaminants is detected here by light scattering analysis on samples stored 7 days at +4°C:



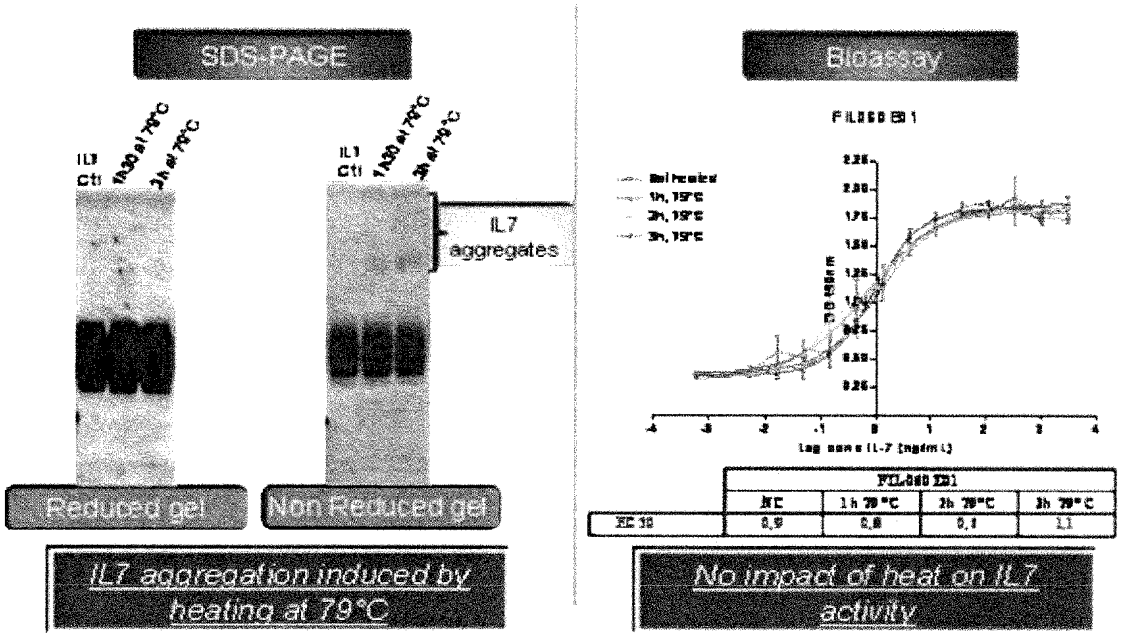
Item	R (nm)	%Pd	MW-R (kDa)	%Int	%Mass
☑ Peak 1	94.7	23.9	141836	64.4	100



Item	R (nm)	%Pd	MW-R (kDa)	%Int	%Mass
☑ Peak 1	2.5	34.2	27	27.7	99.9
☑ Peak 2	40.1	36.5	18969	72.3	0.1

5. Classical bioassay analysis does not discriminate between glycosylated IL-7 batches containing various levels of aggregate contaminants

Purified IL-7 and heat -induced IL-7 aggregates showed different SDS gel profiles on the non-reduced gel but identical biological activity in a cell proliferation assay using the very specific PB-1 cell line, a murine pre-B cell line, which is strictly dependent on IL-7 for growth.



Thus, bioassay analysis is not sufficient to discriminate between properly folded IL-7 and IL-7 compositions containing product related impurities (partly unfolded, improperly folded, and/or aggregated IL-7).

6. Immunogenicity testing in clinical trials, according to the method described in the patent application, discriminates between IL-7 clinical batches with various degrees of contaminants.

Immunogenicity was tested in patients by measuring the presence and titre of anti-IL-7 binding antibodies after 2 to 3 weeks of treatment with IL-7. As noted in the as-filed specification and this declaration, the presence of minimal amount of residual impurities is sufficient to induce immunogenicity.

Source of IL-7	Dose levels used in clinical trials	Number of treated-patients	Antibodies titers	Purity
P01 First GMP batch	3 µg/kg/injection 3 injections per week	1	Presence of high antibodies titer (>1/1200)	Presence of residual impurities
P02 Second GMP batch	3 µg/kg/injection 3 injections per week	8	No antibodies	> 98% Improved purification process

Conclusion:

The present data show that expression in mammalian cells such as CHO or prokaryotic cells such as E. coli does not automatically lead to refolding of IL-7 corresponding to the claimed conformer, that the claimed composition of matter corresponding to the claimed conformer and having at least 98% purity (by weight) differs from commercially available IL-7 preparations, that the claimed composition of matter would reasonably be expected to differ from those disclosed in the Namen et al. and Ho et al. patents and that IL-7 related impurities and other conformers, even in low amounts, trigger anti-IL-7 immunogenicity, which should be carefully monitored during clinical studies.

I declare further that all statements made herein are of our own knowledge true and all statements made on information or belief are believed to be true: further that all statement were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment, or both, under § 1011 of title 18 of the United States code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon. Further Declarant sayeth not.

JULY 15, 2008

Date

Michel MORRE